A Genome-Wide Association Study in Caucasian Women Points Out a Putative Role of the *STXBP5L* Gene in Facial Photoaging

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A genome-wide association study (GWAS) was conducted on 502 French middle-aged Caucasian women to identify genetic factors that may affect skin aging severity. A high-throughput Illumina Human Omni1-Quad beadchip was used. After single-nucleotide polymorphism (SNP) quality controls, 795,063 SNPs remained for analysis purposes. Possible stratification was first examined using the Eigenstrat method, and then the relationships between genotypes and four skin aging indicators (global photoaging, lentigines, wrinkles, and sagging) were investigated separately by linear regressions adjusted on age, smoking habits, lifetime sun exposure, hormonal status, and the two main Eigen vectors. One signal passed the Bonferroni threshold ($P = 1.53 \times 10^{-8}$) and was significantly associated with global photoaging. It was also correlated with the wrinkling score and the sagging score. According to HapMap, this SNP, rs322458, was in linkage disequilibrium (LD) with intronic SNPs of the *STXBP5L* gene, which is expressed in the skin. In addition, it was also in LD with another SNP that increases the expression of the *FBXO40* gene in the skin. These two genes, which were not previously described in the context of aging, may constitute good candidates for the investigation of molecular mechanisms of skin photoaging.

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INTRODUCTION

Similar to other organs, skin ages owing to passage of time. Skin aging is influenced both by inherited intrinsic factors and by extrinsic or environmental factors, such as chronic UV exposure and smoking (Malvy *et al.*, 2000; Yaar and Gilchrest, 2007). Intrinsic aging is an ineluctable process and is due to the genetically determined natural degeneration of the cell functioning and loss of extracellular matrix with age (Yaar and Gilchrest, 1990). Its clinical phenotype on the skin is mainly characterized by fine wrinkles and dry, thin, and pale skin (Fisher *et al.*, 2002; Makrantonaki and Zouboulis, 2007).

The main factor responsible for extrinsic aging of the skin is UVR. UV-induced skin aging or photoaging is defined as the premature occurrence of signs of aging on the skin, and presents with characteristic morphological changes of both the epidermal and dermal compartments (Rabe *et al.*, 2006; Yaar and Gilchrest, 2007). A number of hereditary phenotypic features influence the severity of photoaging, most notably skin color (Kligman and Kligman, 1999; Malvy *et al.*, 2000), and skin phototype (Fitzpatrick, 1988). Individuals with dark phototypes (III–IV) commonly exhibit more ''hypertrophic responses'' such as deep wrinkling, coarseness, and lentigines, whereas fair phototype individuals (I–II) generally show fewer wrinkles with epidermal atrophy, focal depigmentation, as well as dysplastic changes, such as actinic keratosis, nonmelanoma, and melanoma skin cancers (Rabe *et al.*, 2006; Yaar and Gilchrest, 2007; Puizina-Ivić, 2008).

Up to now, the exploration of the genes affecting skin aging has remained limited to *MC1R* gene (Elfakir *et al.*, 2010; Suppa *et al.*, 2011), or to genes involved in genetic pathologies with accelerated skin aging (Rooryck *et al.*,

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Abbreviations: BMI, body mass index; GWAS, genome-wide association study; LD, linkage disequilibrium; SNP, single-nucleotide polymorphism; SU.VI.MAX, SUpplémentation en VItamines et Minéraux AntioXydants

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2008; Soufir *et al.*, 2010). A candidate gene approach has previously established associations between *MC1R* gene variants, particularly loss-of-function variants, with an increased risk of severe photoaging (Elfakir *et al.*, 2010). In addition, a few studies conducted in twin cohorts have explored the associations between environmental factors, skin aging, and gene expression (Plomin *et al.*, 1994; Shekar *et al.*, 2005, 2006; Christensen *et al.*, 2009).

To unravel new genetic associations with skin aging in a systematic way, we have undertaken a genome-wide study on a well-defined sample of Caucasian women from the SU.VI.MAX (*SUpplémentation en VItamines et Minéraux Anti-oXydants* (Antioxidant Vitamin and Mineral Supplementation)) cohort (Hercberg *et al.*, 2004). To the best of our knowledge, no genome-wide association study (GWAS) targeting skin aging in middle-aged women of European-derived ancestry has been previously reported.

RESULTS

Using the Illumina HumanOmni1-Quad BeadChips, we conducted a GWAS by testing associations between single-nucleotide polymorphisms (SNPs) and global skin photoaging on a large sample of French middle-aged women from the SU.VI.MAX cohort. After the various quality-control tests (see Materials and Methods), 795,063 genotyped SNPs were available for 502 women.

Table 1 describes the sample of women according to the severity of photoaging. We also computed the correlations between the age and the outcome variables (Table 2). We found that the correlations with age were all statistically significant ($P<0.0001$): 0.56 for the grade of photoaging, 0.61 for the score of wrinkling and the score of sagging, and 0.27 for the score of lentigines. Similarly, the correlations between the grade of photoaging and the other outcome variables were also statistically significant ($P<0.0001$): 0.78 for the score of wrinkling, 0.66 for the score of sagging, and 0.31 for the score of lentigines; the correlation between the score of wrinkling and the score of sagging reached 0.71 ($P<0.0001$; Table 2).

Our core association analysis focused on genotypic associations obtained using linear regressions, after correction for stratification and nongenetic skin aging factors. Figure 1 presents the distribution of the *P*-values obtained for each SNP along the chromosomes (Manhattan plot). One SNP located on the chromosome 3 (locus 3q13.33), rs322458, passed the Bonferroni threshold (6.28×10^{-8}) with $P=1.53\times10^{-8}$. According to HapMap, this SNP is in linkage disequilibrium (LD) with five SNPs positioned in intronic regions of the *STXBP5L* gene (rs470647, rs612545, rs617332, rs645045, and rs1795413), and with two intergenics SNPs (rs377374 and rs450614; Figure 2). A more refined analysis suggested that the effect was likely recessive. Indeed, when regrouping the individuals according to their grade of skin photoaging, the frequency of the homozygous rs3224587-AA genotype was clearly inversely proportional with photoaging severity (Figure 3): from 28% of homozygous subjects among grade 1 to 4% among grade 5. To further investigate the rs322458 SNP, we assessed its putative impact on each phenotype: lentigines, wrinkling, and sagging. No relationship was found with the lentigines score ($P=0.63$), whereas significant links were found with wrinkling and sagging scores (respectively, $P=5.6\times10^{-5}$ and $P=1.76\times10^{-4}$).

Moreover, bioinformatics databases were investigated for possible associations between SNPs and mRNA expression, regulation (splicing, polyadenylation, and miRNA), and also for putative transcription binding sites. According to Genevar (Nica *et al.*, 2011), the genotype rs470647-AA (rs470647 is in LD with the rs322458; see Figure 2) increases the expression in skin of a neighboring gene, *FBXO40* ($P=6\times10^{-4}$; Figure 4). The rs470647 SNP and *FBXO40* are at a distance of 683 kb.

To further investigate other possible associations, we also computed all the haplotypes based on two SNPs derived from both *STXBP5L* and *FBXO40* genes. Only three haplotypes were strongly associated with photoaging (Figure 4) and they implicated the rs322458 SNP. These haplotypes involved one exonic SNP and one 3′-untranslated region of the *STXBP5L* gene (respectively, rs17740066, $P=6.27\times10^{-9}$ and rs6782033, $P=3.96\times10^{-9}$), and one intronic SNP of the *FBXO40* gene (rs6775899, $P=9.52\times10^{-10}$). The rs17740066 and rs6782033 SNPs were in partial LD with rs322458 ($D'=1$); in other words, the G allele frequency of rs322458 SNP was identical with that of the haplotypes GG (rs322458–rs17740066) and GA (rs322458–rs6782033). Interestingly, the rs17740066 SNP corresponds to the Val855Ile protein variation and rs6782033 SNP corresponds to a putative binding site for a miRNA (hsa-mir-892b; Figure 4). There was no LD between the two SNPs, rs6775899 and rs322458 ($r^2=0.014$ and $D'=0.2$). However, the GA haplotype (rs322458–rs6775899) also exhibited a significant *P*-value ($P=9.52\times10^{-10}$), suggesting it might also be a haplotype of interest.

DISCUSSION

We have described here a GWAS investigating possible associations between SNPs and global skin photoaging. This research yielded an association for the rs322458 SNP connected to the *STXBP5L* gene with severity of skin photoaging, the rs322458-AA genotype being inversely linked with the severity of skin aging. This SNP was also associated with the wrinkle and sagging scores that are defined independently from the grade of photoaging, but it was not associated with the lentigines score, suggesting that: (1) its role in photoaging does not include pigmentary disorders; and (2) molecular mechanisms might be shared by sagging and wrinkling. According to the HapMap database, this SNP is also polymorphic in the Asian and African populations, and thus it would also be worth investigating these populations. As for any GWAS, additional genetic studies will be needed to affirm this association.

Another alias for *STXBP5L* is *LLGL4*, as it is homologous to the Lethal giant larvae (*Lgl*) drosophila gene (Katoh and Katoh, 2004). The protein coded by *STXBP5L* contains five WD40 repeats (or β-transducin repeats) and a C-terminal syntaxin-binding (STXB) domain. *Lgl* regulates epithelial polarity and, when mutated, may lead to tumor-like

Table 1. Description of the population according to photoaging severity

	Photoaging severity						
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5/6 [1]		
	N = 43	*N* = 86	*N* = 174	*N* = 150	*N* = 49	Total, *N* = 502	*P*-value of test
Age (years)	50.1 ± 4.2[2]	54.1 ± 5.0	56.8 ± 5.5	60.9 ± 5.6	62.6 ± 5.2	57.6 ± 6.4	<0.0001[3]
Lifetime sun exposure (score)	5.3 ± 3.4	5.1 ± 3.5	5.2 ± 3.6	5.5 ± 3.5	5.5 ± 3.5	5.3 ± 3.5	0.84[3]
BMI classification							0.49[4]
Normal	28 (8.4)[5]	57 (17.0)	121 (36.1)	94 (28.1)	35 (10.4)	335 (66.7)	
Overweight	9 (7.4)	19 (15.6)	37 (30.3)	45 (36.9)	12 (9.8)	122 (24.3)	
Obese	6 (13.3)	10 (22.2)	16 (35.6)	11 (24.5)	2 (4.4)	45 (9.0)	
Hormonal status							<0.0001[4]
Nonmenopausal	27 (28.7)	24 (25.5)	32 (34.0)	9 (9.6)	2 (2.2)	94 (18.7)	
Menopausal with HRT	9 (3.4)	40 (15.3)	98 (37.4)	92 (35.1)	23 (8.8)	262 (52.2)	
Menopausal without HRT	7 (4.8)	22 (15.1)	44 (30.1)	49 (33.6)	24 (16.4)	146 (29.1)	
Smoking habits							0.61[4]
Never	23 (8.0)	45 (15.7)	100 (35.0)	86 (30.1)	32 (11.2)	286 (57.0)	
Former smoker	15 (9.3)	34 (21.3)	50 (31.2)	47 (29.5)	14 (8.7)	160 (31.9)	
Current smoker	5 (8.9)	7 (12.5)	24 (42.8)	17 (30.4)	3 (5.4)	56 (11.1)	
Eye color							0.21[4]
Blue/gray	14 (10.3)	18 (13.2)	50 (36.8)	36 (26.5)	18 (13.2)	136 (27.2)	
Green/hazel/brown/black	28 (7.8)	68 (18.7)	122 (33.6)	114 (31.4)	31 (8.5)	363 (72.8)	
Hair color at 20 years							0.08[4]
Blond/red	4 (3.7)	20 (18.6)	40 (37.0)	28 (25.9)	16 (14.8)	108 (21.6)	
Light and dark brown/black	38 (9.7)	66 (16.9)	132 (33.8)	122 (31.2)	33 (8.4)	391 (78.4)	
Skin color without tanning							0.78[4]
Fair	35 (9.0)	65 (16.8)	136 (35.0)	113 (29.2)	39 (10.0)	388 (77.8)	
Dark	7 (6.3)	21 (18.9)	36 (32.4)	37 (33.3)	10 (9.0)	111 (22.2)	
History of facial freckles							0.40[4]
No	25 (8.5)	55 (18.7)	104 (35.4)	87 (29.6)	23 (7.8)	294 (58.9)	
Yes	17 (8.3)	31 (15.1)	68 (33.2)	63 (30.7)	26 (12.7)	205 (41.1)	
Suntan intensity							0.71[4]
None/slight/light	23 (7.7)	49 (16.3)	101 (33.7)	96 (32.0)	31 (10.3)	300 (60.1)	
Dark/very dark	19 (9.5)	37 (18.6)	71 (35.7)	54 (27.2)	18 (9.0)	199 (39.9)	
Sunburn event frequency							0.74[4]
None/rare	28 (7.8)	61 (17.0)	123 (34.4)	113 (31.6)	33 (9.2)	358 (71.7)	
Frequent/constant	14 (9.9)	25 (17.7)	49 (34.8)	37 (26.2)	16 (11.4)	141 (28.3)	

Abbreviations: BMI, body mass index; HRT, hormonal replacement therapy.
[1]As a single woman had grade 6, she had been grouped with grade 5 individuals.
[2]Mean ± SD.
[3]Analysis of variance (ANOVA) test.
[4]The χ^2 test.
[5]Frequency and (%): because of possible missing values, the sum of the cell frequencies can be smaller than the total indicated in the top of the columns.

phenotype development. According to bioinformatics analysis (UniProt, 2011), *STXBP5L* seems to be implicated in vesicle trafficking and could have a role in exocytosis ((Katoh and Katoh, 2004; UniProt, 2011). Interestingly, *STXBP5L* has previously been associated with liver fibrosis risk in Caucasians and with chronic hepatitis C infection (Li *et al.*, 2009). *STXBP5L* is expressed in several tissues, including the skin (Safran *et al.*, 2010), and is also expressed in lung carcinoid and germ cell tumors (Katoh and Katoh, 2004).

Bioinformatics database exploration pointed out the possible role of the SNP rs322458 in the skin expression of a neighboring gene, *FBXO40*. Haplotype analysis of the

Table 2. Correlation coefficients between age and outcome variables

	Age	Score of wrinkling	Score of sagging	Score of lentigines	Grade of photoaging
Age	1	0.61	0.61	0.27	0.56
Score of wrinkling		1	0.71	0.31	0.78
Score of sagging			1	0.26	0.66
Score of lentigines				1	0.31
Grade of photoaging					1

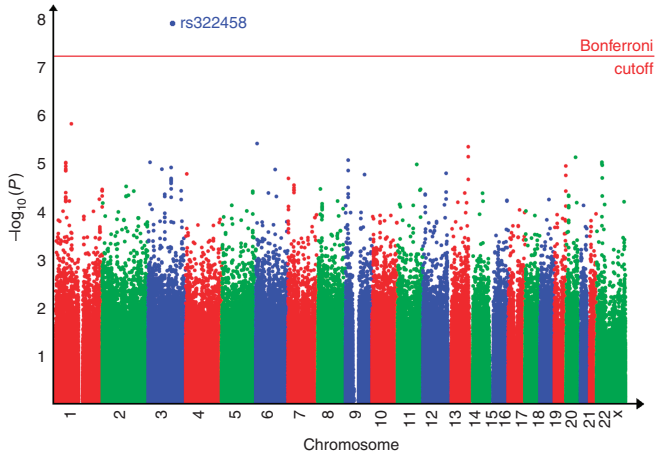

Figure 1. Manhattan plot of the association study with the photoaging score. Distribution of $-\log10(P)$ obtained for the associations tested between the genotypes and skin photoaging, according to Larnier's scale, along the human chromosomes (Manhattan plot).

STXBP5L and *FBXO40* gene region also revealed positive signals ($P \sim 10^{-9}$), bringing up a second hypothesis in which rs3322458 G allele in the dominant mode, possibly in combination with other alleles, might be implicated in the phenotype.

FBXO40 encodes a protein characterized by a 40 amino-acid F-box motif. This gene is expressed specifically in the muscle (Ye *et al.*, 2007), may function as a regulator involved in the postnatal myogenesis (Ye *et al.*, 2007), and has a role in muscle hypertrophy (Shi *et al.*, 2011). F-box proteins are involved in the SCF (Skp, Cullin, F-box containing) complex, known to act as protein–ubiquitin ligases (Skowyra *et al.*, 1997), and a recent study demonstrated that the SCF–F-box40 complex prevented skeletal muscle hypertrophy by limiting the IGF1 pathway in the muscle (Shi *et al.*, 2011).

Both *STXBP5L* and *FBXO40* were not known before for any skin function. How could they affect skin aging? *FBXO40* is linked with the IGF1 pathway known for its role in inflammation, and its direct link with myogenesis could also explain its impact on wrinkling and sagging severity. Knowing that

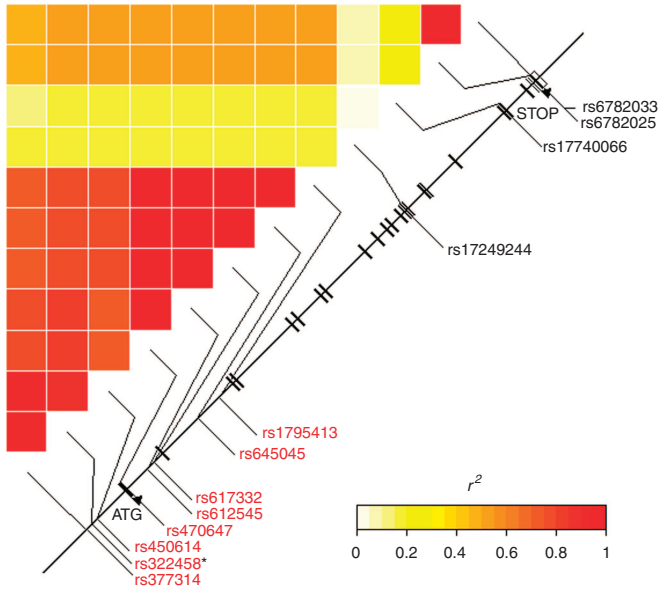

Figure 2. Genetic map of the *STXBP5L* gene. The single-nucleotide polymorphisms (SNPs) in high linkage disequilibrium (LD) with the SNP rs322458 are in red, and the exonic SNPs genotyped in the study are in black. The LD map (providing the r^2 coefficient between SNPs) is given below the genetic map. The SNP rs322458 is flagged with an asterisk (*).

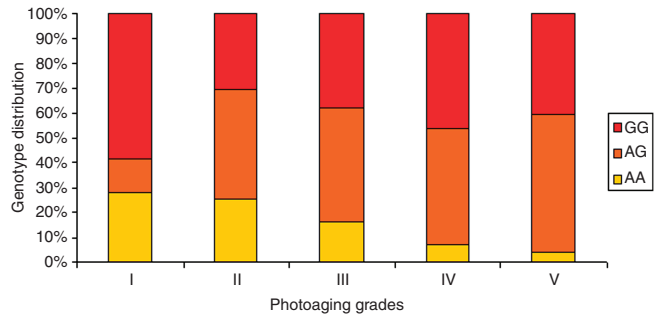

Figure 3. Distribution of the rs322458 genotypes in function of the photoaging severity.

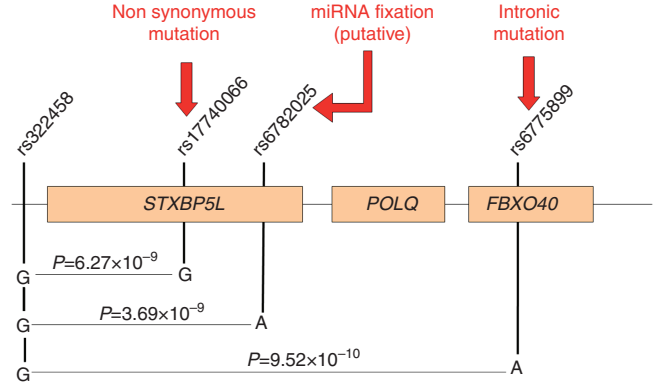

Figure 4. Haplotype analysis. All the two-single-nucleotide polymorphism (SNP) haplotypes of the region were computed using the Shape-IT software. Associations were computed, and the figure presents the three best signals obtained, which all involve the SNP rs322458.

photoaging is intimately associated with the occurrence of dysplastic skin changes, such as actinic keratosis as well as nonmelanoma and melanoma skin cancer, it is striking to see that *STXBP5L* has been linked to cancer (Katoh and Katoh, 2004; Li *et al.*, 2009). Therefore, the search for gene polymorphisms involved in photoaging may also help to identify risk factors for skin carcinogenesis.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted to investigate skin aging in the context of the SU.VI.MAX cohort, a longitudinal cohort study, conducted in French middle-aged adults (Hercberg *et al.*, 1998). The protocol was approved by the Hospital Medicals Ethics Committee of Paris-Cochin (CCPPRB no. 706) and the "*Commission Nationale de l'Informatique et des Libertés*" (CNIL no. 334641). The study was conducted according to the Declaration of Helsinski Principles. All participants gave their written, informed consent. The SU.VI.MAX cohort included 13,017 volunteers who were representative of the French adult middle-aged population for most sociodemographic features (Hercberg *et al.*, 2004).

This study was conducted in the autumn/winter of 2002–2003. All women living in the Paris area were requested to participate in this research. Among them ($n=2,257$), 570 women, aged 44–70 years, agreed to take part in this study and provided informed consent. The participants were asked to follow specific skin care instructions; notably, application of detergents or cosmetics to the face was not authorized for at least 12 hours before the study visit. On the day of the visit, they were first asked to complete a self-administered questionnaire related to lifetime sun exposure behavior. Subsequently, three standardized, high-resolution digital images ($2,008 \times 3,032$ pixels) of the face were taken for each participant (one frontal view of the face and one of each profile), using a Kodak DCS 760 digital camera with a 105 mm camera lens (Kodak, Paris, France). The camera was mounted on a monopod and a specifically developed chair was used to allow standardized positions of the camera with respect to the face. Lighting conditions were standardized by means of two symmetrical lamps, which provided a continuous daylight spectrum, placed at 45° to each side of the face. Finally, a blood sample was collected for genetic analysis.

Assessment of skin aging features

The facial photographs was examined for each woman by a dermatologist, and the severity of global skin photoaging was rated using a six-grade ordinal scale (Larnier *et al.*, 1994), each grade being depicted by three reference photographs that illustrate the diversity and range of pigmentation disorders, wrinkling, and sagging. In addition, the severity of 12 age-related skin features was also assessed on forehead and on cheeks using specific ordinal photographic scales (Morizot *et al.*, 2002).

Outcome variables: phenotypes analyzed

The primary outcome variable is the global photoaging grade (1–6) and the secondary outcomes variables are the three independent scores: wrinkling, sagging, and lentigines scores. On the basis of the 12 age-related skin features, the global severity of wrinkling, sagging, and solar lentigines was estimated by three scores built using principal component analysis and linear regression methods (Jobson, 1992). Then, each individual's score values were transformed to fit a range between 0 and 10.

The solar lentigines score is computed as follows: $1.25\times$ severity on cheeks $+1.25\times$ severity on forehead (with grade $0=0$, grade $1=1$, grade $2=2$, grade $3=3$, and grade $>3=4$ for each skin area). The sagging score is based on four features: 0.87 when presence of bags under the eyes $+0.78\times$ severity of nasolabial fold (with grade $<3=1$, grade $3=2$, grade $4=3$, and grade $>4=4$) $+0.93\times$ severity of tissue slackening $+1.07\times$ severity of drooping eyelids (with grade $<3=1$, grade $3=2$, and grade $>3=3$ for the two preceding features). Finally, the wrinkling score is computed using the six remaining features: $-0.64+0.42\times$ severity of wrinkles above the upper lip (with grade $0=0$, grade $1=1$, grade $2=2$, grade $3=3$, and grade $4=4$) $+0.64\times$ severity of wrinkles under the eyes (with grade $<3=0$, grade $3=1$, grade $4=2$, and grade $5=3$) $+0.70\times$ severity of fine lines on cheek (with grade $0=0$, grade $1=1$, and grade $2=2$) $+0.44\times$ severity of furrows between eyebrows $+0.54\times$ severity of crow's feet $+1.06\times$ severity of coarse wrinkles on cheek (with grade $<2=0$, grade $2=1$, grade $3=2$, grade $4=3$, and grade $5=4$ for the three preceding features).

Covariables used for the statistical analysis: general and phenotypic data

To focus more specifically on the genetic factors affecting skin aging, several characteristics known to affect aging had to be taken into account: age (in years), body mass index (BMI; in $kg\,m^{-2}$), smoking habits (never, former, and current), and hormonal status (nonmenopausal, menopausal with hormone replacement therapy, and menopausal without hormone replacement therapy). BMI was categorized as underweight/normal (BMI $<25\,kg\,m^{-2}$), overweight ($25\leqslant BMI<30\,kg\,m^{-2}$), or obese (BMI $\geqslant 30\,kg\,m^{-2}$) according to the World Health Organization (WHO) recommendations (WHO, 1995). In addition, phenotypic data such as natural hair color at the age of 20 years, eye color, skin color in winter, sunburn event frequency, suntan intensity, and history of facial freckles were also collected. Moreover, lifetime sun exposure intensity was estimated by a score based on data collected by a self-reported questionnaire. This score is a linear combination of five items weighted according to their relative contribution to the score: voluntary sun exposure, exposure of the body and the facial skin, exposure during the hottest hours of the day, intensity of self-reported lifetime sun exposure, and consideration for sunbathing. The design, validation, and description of this score have been described previously (Guinot *et al.*, 2001).

Genotyping method

The 529 women were genotyped using Illumina Infinium HumanOmni1-Quad BeadChips (Illumina, San Diego, CA) that contain 1,140,419 markers. Genomic DNA (250 ng) was whole-genome amplified, fragmented, denatured, and hybridized on prepared HumanOmni1-Quad BeadChips for a minimum of 16 hours at 48 °C. Nonspecifically hybridized fragments were removed by washing, and the remaining specifically hybridized DNA was fluorescently labeled by a single base extension reaction and detected using a IScan scanner (Illumina). Normalized bead-intensity data obtained for each sample were loaded into GenomeStudio software (version 1.6.3; Illumina), which converted fluorescence intensities into SNP genotypes. For the analysis, we considered only SNPs, consequently

excluding the copy-number variations that represented 91,706 markers on the HumanOmni1-Quad BeadChips. Moreover, 2,182 SNPs were removed because they were located on the Y chromosome and they could not be analyzed as the population was composed of women.

Quality control

Using the GenomeStudio software (version 1.6.3; Illumina), we analyzed the crude genotyping data, and SNPs were filtered according to the following parameters. First, nine samples with a call rate (percentage of SNPs genotyped by sample) of <95% in the Illumina clusters were removed. Second, the SNPs with a call frequency (percentage of samples genotyped by SNP) of <99% were reclustered. Third, after reclustering, samples with a call rate <98% were deleted. This method has been already used in several studies (Le Clerc *et al.*, 2009; Limou *et al.*, 2009, 2010). The clustering step can create SNP genotyping errors, which can be prevented by following the Illumina procedure (http://www.illumina.com/Documents/products/technotes/technote_infinium_genotyping_data_analysis.pdf).

This method evaluates the quality of the newly created clusters according to several criteria, which can be manually checked and corrected as necessary. In total, after all the quality control steps were carried out, 56,479 SNPs with a call frequency of <98% (2% of missing data) were excluded. This procedure ensures reliable genotyping data with little missing data. Hardy–Weinberg equilibrium analysis was performed for each SNP in each group by using an exact statistical test implemented in PLINK software (Purcell *et al.*, 2007). Deviation from Hardy–Weinberg equilibrium in a group of patients suggests an error in genotyping. Thus, 3,866 SNPs, which were not in the Hardy–Weinberg equilibrium ($P<1.0\times10^{-3}$), were rejected in this way. We removed 191,123 SNPs with minor allele frequency <1% to avoid error of genotyping, leaving a total of 795,063 SNPs.

Identification of population stratification

To correct for possible population stratification, genotypes were analyzed using EIGENSTRAT utility of the EIGENSOFT package version 2.0 (Price *et al.*, 2006). The two first pass with the Eigenstrat software pointed out 18 outliers, which were removed from further analyses. Then, a third pass without outliers was performed to determine the Eigen vectors. In the statistical analysis, we used the top two Eigen vectors as covariables to correct for population substructure in the association analyses (Price *et al.*, 2006).

Statistical analysis

Of the 570 women who participated in the study, 68 were excluded from the analysis: 18 had a history of recent antiaging invasive procedures and 10 were observably non-Caucasian. In addition, one sample was removed because of insufficient DNA concentration, 12 samples were removed because the DNA was damaged, and nine samples were removed after quality control. Furthermore, 18 outliers appeared during the stratification analysis. Thus, the population investigated for our genome-wide association study was composed of 502 individuals.

The population was first described according to the severity of photoaging, using a series of analyses of variance for quantitative variables and using χ^2 tests for qualitative variables. In addition, Kendall rank correlation coefficients were calculated between age and each outcome variable, and between each pair of outcome variable (Armitage, 1971). Then, for the remaining 795,063 SNPs and 502 women, the associations between the genotypes and skin photoaging were tested. The statistical analysis was performed by a multivariate linear regression (PLINK software; Purcell *et al.*, 2007) in the genotypic mode, taking as covariables the two first Eigenstrat principal components and the potential confounding factors (smoking habits, BMI, hormonal status, lifetime sun exposure intensity, and age). The *P*-values were adjusted by the Bonferroni correction (statistical threshold $=6.28\times10^{-8}$). Finally, for the secondary outcome variables, additional analyses were performed using the same methodology.

Haplotype inference and LD

Haplotype inference was obtained using the rapid and accurate Shape-IT algorithm (Delaneau *et al.*, 2008, 2012). Then, for each SNP exhibiting a significant association, we looked for other SNPs in LD ($r^2>0.8$) in the HapMap population of Western European ancestry (CEU, HapMap data Release 24/phase II November 2008, on NCBI B36 assembly, dbSNP126; available at: http://www.hapmap.org) to identify the genes possibly involved with the associations. A SNP was assigned to a gene if it was located in the gene or in the 2-kb flanking regions (potential regulatory sequence); otherwise, it was considered intergenic. It is important to note that LD in HapMap population of Western European ancestry is very similar in our group of patients.

Bioinformatics exploration

To further explore the signals observed by the GWAS by using bioinformatics exploration we tried to look for modifications in mRNA expression levels (Yang *et al.*, 2010; Nica *et al.*, 2011; Dixon *et al.*, 2007; Zeller *et al.*, 2010), splicing (NetGene2, http://www.cbs.dtu.dk/services/NetGene2/), polyadenylation regions (polyAH, http://linux1.softberry.com/berry.phtml?topic=polyah&group=programs&subgroup=promoter and polyApred, http://www.imtech.res.in/raghava/polyapred/submission.html), transcription factor binding sites (SignalScan, http://www-bimas.cit.nih.gov/molbio/signal/, TESS, http://www.cbil.upenn.edu/cgi-bin/tess/tess?RQ=WELCOME, and TFSearch, http://www.cbrc.jp/research/db/TFSEARCH.html, derived from TRANSFAC database), and miRNA genes or miRNA targets (miRBAse, http://www.mirbase.org/, miRTarBase, http://mirtarbase.mbc.nctu.edu.tw/, MicroCosm Targets, http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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